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Feral Swine *Leptospira* Seroprevalence Survey in Hawaii, USA, 2007–2009

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Summary

Leptospirosis is considered the most widespread of zoonotic diseases. It was a notifiable disease in the United States until 1995 and was reinstated to the list of nationally notifiable diseases in 2014. During the time of national surveillance, Hawaii consistently led the nation in reported annual incidence rates. Leptospirosis has remained a reportable disease in Hawaii. Significant changes have been documented since the early 1970s in the predominant serogroup infecting humans in Hawaii: infections due to *Icterohaemorrhagiae* have declined while infections due to *Australis* have increased. A recent study from Hawaii demonstrated that *Australis* was an uncommon infecting serogroup for small mammal hosts. Swine have not been previously studied in Hawaii but are well-recognized maintenance hosts for leptospires belonging to the *Australis* serogroup. This study was undertaken to assess the prevalence of *Leptospira* antibody in feral swine in Hawaii. From January 2007 through December 2009, blood samples were collected opportunistically from feral swine. Using the microscopic agglutination test, we found antibody titres 1 : 100 to leptospires in 272 (33.8%) of 804 feral swine. The most frequently reacting serovars to the swine sera were *Icterohaemorrhagiae* (*Icterohaemorrhagiae* serogroup) (41.5%) and Bratislava (*Australis* serogroup) (33.8%). The high seroprevalence and presumptively infecting serovars suggest a link between swine and human infection.

Keywords

Feral swine; leptospirosis; Hawaii; survey

Introduction

Leptospirosis is considered the most widespread zoonotic disease globally, and while uncommonly diagnosed in the United States, it is a notable re-emerging infectious disease in developing countries (World Health Organization, 2011). It was nationally notifiable in the United States until 1995 and was reinstated to the list of nationally notifiable diseases in

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2014 (Centers for Disease Control and Prevention, 2015). During the time of national surveillance, Hawaii consistently led the nation in reported annual incidence rates (Katz et al., 2011). The reported mean annual incidence rate of human leptospirosis in Hawaii from 1974 through 1998 was 2.76 per 100 000. This is approximately 100 times higher than the 0.02–0.05 per 100 000 annual incidence rate reported nationally from 1974 through 1994 (Katz et al., 2002). Leptospirosis has remained a reportable disease in Hawaii, and significant changes have been documented in the infective serogroup among human cases from 1974 through 2008: while infections due to serogroup Icterohaemorrhagiae have been on the decline, those due to Australis have been on the increase (Katz et al., 2011). The observed shift may reflect the influence of different animal hosts, the effects of climatic and land use changes, or both. A recently published small mammal prevalence survey of rats, mice and mongooses in Hawaii showed a preferential association of *Leptospira* serogroups with particular animal hosts: *Rattus* spp. with serogroup Icterohaemorrhagiae, mice with serogroup Ballum and mongooses with serogroup Sejroe; infections with Australis serogroup were rare (Wong et al., 2012). Swine are commonly infected with *Leptospira* and have a well-described ‘maintenance host’ role for serogroup Australis, serovar Bratislava (Faine et al., 1999; Ellis, 2012). A recently published US feral swine *Leptospira* seroprevalence survey from 28 states demonstrated 13% of swine with MAT titres 1 : 200. The two most commonly identified presumptively infecting serovars were Pomona and Bratislava (Pedersen et al., 2015). The current study was undertaken to assess the prevalence of *Leptospira* antibodies in feral swine in Hawaii.

Materials and Methods

Blood samples were collected opportunistically from feral swine on the islands of Oahu, Hawaii, Kauai and Maui from January 2007 through December 2009 by hunters and wild-life biologists performing swine removal for environmental management purposes. Samples were obtained by cardiac puncture in serum separator tubes. After centrifugation, sera were tested for antibodies to Classical Swine Fever virus, *Brucella* and Pseudorabies virus as part of an ongoing surveillance programme. Remaining sera were stored at -18°C .

Serum samples were analysed with the microscopic agglutination test (MAT) at the US Centers for Disease Control and Prevention using a panel of 20 *Leptospira* serovars (Australis, Bratislava, Autumnalis, Ballum, Bataviae, Canicola, Celledoni, Cynopteri, Djasiman, Grippotyphosa, Borincana, Icterohaemorrhagiae, Mankarso, Javanica, Georgia, Pomona, Alexi, Pyrogenes, Wolfii and Tarassovi). A titre 1 : 100 was considered positive (Ellis, 2012). The serovar with the highest titre was considered the presumptively infecting strain. If two or more serovars had the same high titre, the identification of the presumptively infecting strain was labelled indeterminate.

Frequencies and tests for difference were calculated using Epi Info Version 7.0.9.2 (CDC, Atlanta, GA, USA). *P*-values ≤ 0.05 were considered statistically significant; all tests were two-tailed. This research was approved by the University of Hawaii’s Institutional Animal Care and Use Committee.

Results

Of 804 feral swine tested, 272 (33.8%) had MAT titres 1 : 100. Forty-eight (17.6%) of the 272 had titres 1 : 800 reflecting probable acute infection (Faine et al., 1999; Pedersen et al., 2015). The most frequently identified presumptively infecting serovars were Icterohaemorrhagiae [113 (41.5%) of 272] and Bratislava [92 (33.8%) of 272] (Table 1). Most specimens (797 of 804) were collected from the island of Hawaii (419) or Oahu (378).

For 2009 only, data were available on individual swine for island captured, gender and age. Of 144 swine tested in 2009, 49 (34.0%) were positive. Seroprevalence on the island of Hawaii was 40.5% (15 of 37) compared with 31.1% (33 of 106) on Oahu [Fisher's exact test (FET), $P = 0.32$]. Of 136 animals with known sex, 24 (32.4%) of 74 males and 22 (35.5%) of 62 females had antibodies to *Leptospira* (FET, $P = 0.72$). Of 104 swine whose age was estimated, 27 (38.0%) of 71 adults and 6 (18.2%) of 33 sub-adults and juveniles had antibodies to *Leptospira* (FET, $P = 0.07$).

Discussion

Our high (33.8%) *Leptospira* seroprevalence is similar to that reported from a recently published Florida study. Using the same MAT-positive test result definition, Chatfield et al. (2013) found a 33% seroprevalence in feral swine in Florida. A recently published national study by Pedersen et al. (2015) reported a seroprevalence of 13% among feral swine from 28 US states sampled opportunistically, but used an MAT titre 1 : 200 to define a positive test result. Using this definition, our seroprevalence is 23.6% (190 of 804), almost twice that seen nationally. The high seroprevalence seen in Hawaii and Florida may be related to their tropical climate. Recent human outbreaks of leptospirosis have been reported from both states (Gaynor et al., 2007; Stern et al., 2010). Using the same definition of positive (MAT titre 1 : 100), our *Leptospira* seroprevalence was higher than those reported from feral swine in Italy (6%) (Ebani et al., 2003), Germany (18%) (Jansen et al., 2007) and Sweden (3.1%) (Boqvist et al., 2012). A recent study from Australia reported a seroprevalence of 53% among feral swine (Ridoutt et al., 2014), but it used a lower threshold to define positive results (MAT titre 1 : 50). Two studies from tropical South American locales demonstrated much higher *Leptospira* seroprevalence. Using the same definition of positive (MAT titre 1 : 100), *Leptospira* seroprevalence among farmed pigs in Brazil (Ramos et al., 2006) and Colombia (Calderón et al., 2014) was 66.1% and 55.9%, respectively.

While positive results do not equal current infection, swine are known to be maintenance hosts for *Leptospira*, and the high overall seroprevalence reflects high potential for environmental contamination with subsequent human exposure and infection during the lifespan of the animal (Pedersen et al., 2015). In addition, our prevalence measurement may be an underestimate of the true prevalence as infected swine may have MAT titres below 1 : 100 for strains such as Bratislava for which a maintenance host relationship exists (Ellis, 2012; Pedersen et al., 2015).

The two most commonly identified presumptively infecting serovars, Bratislava (serogroup Australis) and Icterohaemorrhagiae (serogroup Icterohaemorrhagiae), were also commonly

found in both the US national and Florida feral swine surveys (Chatfield et al., 2013; Pedersen et al., 2015). Bratislava was the most commonly identified presumptively infecting serovar in Florida followed by Icterohaemorrhagiae (Chatfield et al., 2013), while Bratislava was the second most commonly identified serovar (following Pomona) in the US national study (Pedersen et al., 2015). Bratislava was also the most commonly identified serovar in the Italian and Swedish studies (Ebani et al., 2003; Boqvist et al., 2012) (followed by Icterohaemorrhagiae) and the second most common serovar in the German study (Jansen et al., 2007).

The MAT is considered the gold standard serological assay for leptospirosis. However, due to cross-agglutination and cross-reactions between both serogroups and serovars, the MAT only allows for the presumptive identification of infecting serogroups and serovars (Hartskeerl and Wagenaar, 2015). Definitive identification requires culture of isolates (Levett, 2003; Haake and Levett, 2015). However, an earlier study in Hawaii demonstrated a relatively good agreement ($\kappa = 0.71$) between the infecting serogroup identified through culture isolation and the MAT identified presumptively infecting serogroup (Katz et al., 2003).

Hawaii has documented an increase in the feral swine population with encroachment on residential areas (Katz et al., 2011). Our findings suggest that feral swine may be linked to the increasing trends in Australis infections in humans. Continued research is needed to identify *Leptospira* culture isolates from feral swine samples to better assess the link between swine and human disease.

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Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Impacts

- Hawaii has the highest incidence of leptospirosis in the United States.
- The predominant *Leptospira* serogroup infecting humans in Hawaii has changed since the early 1970s from Icterohaemorrhagiae to Australis. The Australis serogroup is uncommon among small mammal hosts in Hawaii.
- This study, reporting the results of the first feral swine *Leptospira* antibody survey undertaken in Hawaii, provides evidence linking swine to human infection.

Table 1.

Presumptively infecting *Leptospira* serovar of feral swine, identified by microscopic agglutination test (MAT), Hawaii, USA, 2007–2009

Serovar	No. (%)
Icterohaemorrhagiae	113 (41.5)
Bratislava	92 (33.8)
Autumnalis	12 (4.4)
Djasiman	7 (2.6)
Georgia	3 (1.1)
Australis	2 (0.7)
Pyrogenes	1 (0.4)
Pomona	1 (0.4)
Celledoni	1 (0.4)
Indeterminate	40 (14.7)
Total	272 (100)

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